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Reconnaissance of dioxin-like and estrogen-like toxicities in sediments of Taean, Korea-seven years after the *Hebei Spirit* oil spill

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HIGHLIGHTS

- Dioxin-like toxicities were found in sediments affected by *Hebei Spirit* oil spill.
- Fractionized samples could not explain toxicities observed in whole extracts.
- Toxicity interactions of oil components are suspected in sediment samples of HSOS.
- Follow-up of mechanism-based bioassays combined with chemical analyses is required.

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ABSTRACT

Oil spills near the coastlines may damage marine and intertidal ecosystem. Constituents of the oil have been reported to cause toxic consequences mediated by aryl hydrocarbon receptor (AhR), and estrogen receptor (ER). In the present study, AhR– and ER–mediated toxicities of coastal sediments of Taean were investigated seven years after *Hebei Spirit* oil spill (HSOS). Sediment samples were collected on June and October 2014 from seven locations along the Taean coastline, where signs of oil spill were detected. Sediment samples were extracted in Soxhlet extractors and further processed through activated silica gels to separate into four fractions; F1 (saturate hydrocarbons), F2 (aromatic hydrocarbons), F3 (resins and polar compounds), and F4 (residues). ER–mediated and AhR–mediated potencies (% $E2_{max}$ and % $TCDD_{max}$) of each fraction were determined using MVLN cells and H4IIE-*luc* cells, respectively. F2 and F3 fractions of Sinduri 1, Sinduri 2, and Sogeunri 1 samples showed greater AhR–mediated potencies (up to 107% $TCDD_{max}$). Chemical analysis revealed that PAH components are correlated with AhR–binding activities. The % $E2_{max}$ results varied by sample: While there was no noticeable induction of ER –dependent responses (<45%), some aromatics fractions (F2) exhibited the highest ER–mediated responses. Compared with previous reports from the same sites, both AhR–mediated and ER–mediated potencies have decreased over time. Nevertheless, AhR–mediated potencies could be identified in the environmental samples even after 7 years of the incident. Therefore, possible ecosystem implications of these findings should be further investigated.

1. Introduction

The *Hebei Spirit* oil spill (HSOS), which occurred in the Yellow Sea near Taean coastline (Fig. S1) on December 2007, was the largest oil spill accident in Korea to date. Approximately 10,900 tons of crude oil were spilled into the sea impacting more than 375 km shoreline of the west coast of Korea (Yim et al., 2012). One of the largest coastal mud flat in Korea is located along this coastline. Hence significant damages to the diverse benthic organisms

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and associated ecosystem receptors were of concern. Following extensive cleanup and restoration efforts, roughly 20% of oil was removed within one year from the shoreline (Yim et al., 2012), and more in following years. However, stranded oils are still found in sediments around the spill site, and therefore continuous monitoring of the impacted area has been carried out.

Crude oil is a complex mixture of polycyclic aromatic hydrocarbons (PAHs), heavy metals, and other chemicals. In addition, spilled oil may undergo weathering processes such as evaporation, dissolution, biodegradation, and photodegradation (Yim et al., 2011). Through these physical, chemical, and biological weathering processes, compositions of spilled oil are changed (Boehm and Flest, 1982; Hong et al., 2012). Hydrocarbons that have been measured in sediments along the Taean coastal area clearly show complex chemical compositions of the oil spill (Yim et al., 2011; Hong et al., 2012). Such complexity of composition makes it difficult to predict and evaluate the toxicity of stranded oil remnants in the environment following oil spills. Therefore, multiple approaches to identify toxic effects of stranded oils on ecosystem are necessary.

Most planar compounds, such as polycyclic aromatic hydrocarbons (PAHs) and halogenated aromatic hydrocarbon (HAH), are metabolized via the aryl hydrocarbon receptor (AhR) pathway (Schmidt and Bradfield, 1996). Therefore, the binding potencies of chemicals to AhR have been utilized as biomarker assays for AhR ligands (Safe et al., 1989). One such assay system is the H4IIE-*luc* cell line-based assay. This cell line has been used widely to assess AhR-mediated potencies of PAHs and HAH mixtures in environmental samples for decades (Tillitt et al., 1991; Willett et al., 1997). However, discrepancy between chemical analyses and AhR-mediated potencies has been often reported in environmental samples (Zacharewski et al., 1989; Schmitz et al., 1996), which might be due to complexity of chemical compositions and associated toxicity interactions.

Continuous efforts have been made to monitor AhR-mediated potencies or biological responses of sediments after the HSOS (Hong et al., 2012, 2016; Jung et al., 2011). Cytochrome P4501A (CYP1A) induction mediated by AhR has been widely used as a biomarker of exposure to aromatic hydrocarbons in aquatic organisms (Goksøyr and Förlin, 1992; Bucheli and Fent, 1995). Concentrations of PAHs in fish decreased to background levels, but significant increases in PAH metabolites and hepatic CYP1A induction were still observed a year after the HSOS (Jung et al., 2011).

In addition, endocrine disrupting effects have been reported on crude oils (Michel and Hayes, 1999; Vrabie et al., 2010, 2011; Kim et al., 2016). Indeed, PAHs, major components of oil, are known to affect estrogen receptor (ER)-mediated pathways via crosstalk between ER and AhR (Swedenborg and Pongratz, 2010). Oils could induce estrogenic effects by disrupting specific estrogen receptor binding in yeast estrogen screening test and in mammalian cell line assays (Vrabie et al., 2010). In addition, oil affected sediments could cause steroidogenic alteration in a human adrenal (H295R) cells (Ji et al., 2011). However, the information on estrogen-mediated toxic effects of oil contaminated sediments is generally very limited.

In the present study, we monitored AhR− and ER−mediated toxicities of the sediment samples collected from the coastline near the HSOS site, seven years after the incident. Sediment samples were collected in June and October 2014 from seven locations where residual oils were detected. The results of this study will provide information regarding the AhR− and ER-mediated potencies of sediment after several years of oil spill, and will help the local government and relevant governmental agencies design necessary long-term monitoring and follow-up measures for HSOS and similar oil spill accidents worldwide.

2. Materials and methods

2.1. Sampling location

In Sinduri beach June 2014, where large amount of stranded oil was found, core samples (RO) were collected. After 4 months, in October 2014, sampling was conducted at the same point (Sinduri beach, SD1) and the backshore zone of SD1, i.e., SD2. Samples were also collected from two nearby beaches affected by the HSOS, i.e., Sogeunri mudflat (SG1−3) and Euihangri mudflat (EH1). The collected samples were immediately stored at −20 °C until analysis. More information on sampling sites is shown in Fig. S1 and Table S1 of Supplement.

2.2. Soxhlet extraction

Sediment extraction was conducted following Hong et al. (2015). Briefly, 30 g of wet sediments were mixed with anhydrous sodium sulfate (Sigma Aldrich, Saint Louis, MI, U.S.A.) to remove water. Samples were extracted with 250 mL of dichloromethane (DCM, Burdick and Jackson, Muskegon, MI, U.S.A.) in a Soxhlet extractor for 16 h and concentrated by rotary evaporation. Activated copper (Merck, Darmstadt, Germany) and sodium sulfate (Sigma Aldrich) were added to remove elemental sulfur, residue water, and copper powder respectively and then concentrated to 6 mL under a gentle stream of nitrogen.

2.3. Silica gel fractionation

An aliquot of 2 mL of raw extract (RE) from each sample was passed through 8 g of activated silica gel in a packed glass column for fractionation following our protocol described previously (Hong et al., 2015). The first fraction (F1) was eluted with 30 mL of hexane containing saturate hydrocarbons. The second fraction (F2), i.e., aromatic fraction, was collected by elution with 50 mL of 20% (v/v) DCM in hexane. Resins and polar compounds (F3) were eluted in 50 mL of 60% (v/v) DCM in acetone (Burdick and Jackson). The residue of RE sample (F4) was collected by elution with 40 mL of acetone. All eluents were concentrated to 2 mL under gentle stream of nitrogen for use in the bioassay (Hong et al., 2015).

2.4. Sediment PAH concentration measurement

A total of 33 parent−PAHs and alkyl−PAHs were measured using an Agilent 7890 gas chromatograph (GC) coupled to a model 5975C mass−selective detector (MSD, Agilent Technologies, Anondale, PA, U.S.A.) according to a previously described method (Hong et al., 2012). The method detection limits (MDL) for individual PAHs ranged from 0.1 to 0.5 ng/g dry weight.

2.5. H4IIE-luc bioassay

H4IIE-*luc* bioassay was performed according to Khim et al. (1999) with slight modifications. Cells were seeded into the 60 interior wells of 96- well microplates (250 μL per well) at a density of 8.0×10^4 cells/mL. Exterior wells of each plate were filled with equal volume of phosphate buffered saline (PBS) to maintain humidity for cell growth. After overnight incubation, control and test wells were dosed with 2.5 μL of standards and test chemicals dissolved in DMSO. DMSO did not exceed 0.1% (v/v) of cell culture media. Standards (TCDD) and test chemicals were prepared in six and five concentration ranges via serial dilutions, respectively; standards were diluted by 3-fold (30, 10, 3.3, 1.1, 0.33, and 0.11 pM) and test chemicals were diluted by 2-fold (100, 50, 25, 12.5, and 6.25%). All treatments were tested in triplicates. Luminescence was

measured after 72 h of exposure with Tecan infinite® 200 microplate reader (Männedorf, Switzerland). Exposure time was determined following previous studies (Hong et al., 2012, 2015; Lee et al., 2013). R^2 values for the dose-response curves of the standard were above 0.98. The intensity of luminescence was converted to % $TCDD_{max}$. Specifically, responses of the bioassay, expressed as mean relative units of luminescence were converted to a percentage of the maximum response (% 2,3,7,8-$TCDD_{max}$) for a standard containing 30 pM (= 100% $TCDD_{max}$). A separate WST-1 cell proliferation assay (Roche Applied Science, Mannheim, Germany) was performed to ensure that tested concentrations were not lethal to the cells. WST−1 cell proliferation assay confirmed that concentrations used in this study were not cytotoxic (data not shown).

2.6. MVLN-luc bioassay

MVLN-*luc* bioassay was performed using the same methods as H4IIE-*luc* bioassay, except for seeding density of the cells (1.25×10^5 cells/mL), and concentrations of the standard (17β-estradiol, E2). Standards and test chemicals were prepared in six and five concentration ranges via serial dilutions, respectively; standards were diluted by 3-fold (370, 123, 41, 13.7, 4.6, and 1.5 pM) and test chemicals were diluted by 2 fold (100, 50, 25, 12.5, and 6.25%). DMSO did not exceed 0.1% v/v of cell culture media. Test and control wells (250 μL per well) were dosed with 2.5 μL of the standard and test chemicals. All treatments were tested in triplicate. Luminescence was measured after 72 h of exposure with Tecan infinite® 200 microplate reader. Only the results from experiments with R^2 values above 0.86 were used. The intensity of luminescence was converted to % $E2_{max}$ (13.7 pM = 100% $E2_{max}$) using the same calculation method as described for % $TCDD_{max}$. A separate WST-1 cell proliferation assay confirmed that concentrations in this study were not cytotoxic (data not shown).

2.7. Statistical analysis

Principal components analysis (PCA) was performed to screen the pattern of chemical concentrations of sediment samples, and also used to infer relationships between chemical concentrations and biological effects. All statistical analyses were conducted using R software (ver3.2.1) (R Development Core Team, 2008).

3. Results and discussion

3.1. Chemical analysis

All samples contained detectable concentrations of total PAHs, with the greatest concentration measured in RO (962.14 ng/g), followed by SG1 (223.07 ng/g), SG2 (67.62 ng/g), SG3 (41.54 ng/g), SD1 (16.23 ng/g), SD2 (1.25 ng/g) and EH1 (0.42 ng/g) (Table 1). PAHs have been measured from the sediments collected from the same locations since 2007. A range between 4.7×10^3 and 1.76×10^5 ng/g of total PAHs has been measured in the sediments from the contaminated sites of Taean (Yim et al., 2011; Hong et al., 2012, 2015), which is much higher than the reported average levels of 13.7 ng/g (16 PAHs) to 17.7 ng/g (24 PAHs) measured prior to the HSOS (Yim et al., 2007). The concentrations measured are roughly comparable to those measured from sediments affected by other major oil spills, including the *Prestige* oil spill (5.0×10^3–2.06×10^5 ng/g; Puente et al., 2009), T/V *Erica* oil spill (618-218 ng/g; Tronszyński et al., 2004), and South Caspian Sea (2.99×10^3 ng/g; Tolosa et al., 2004). Overall, reports indicate that water quality (oil concentrations in seawater and pore water) and chemical concentrations in the area's biota have returned to background levels (Kim et al., 2010; Jung et al., 2011). However, benthic communities, and sediment quality have not recovered fully (Xia et al., 2011; Hong et al., 2012; Yu et al., 2013; Kang et al., 2016). Our results are consistent with previous studies; sites with high overall total PAHs still exist, and there are variations among sampling locations, even among nearby sites. Nevertheless, as can be seen in the dramatic differences in the concentrations of PAHs from the sampling locations over time, the contaminant levels have been continuously decreasing in the area.

Over 90% of PAHs detected in the sediments were alkyl−PAHs (Table 1). A closer examination of the alkyl-PAH composition (C1 to C4 alkyl−PAHs) in each sample revealed that samples could be grouped by their chemical composition (Fig. 1 and Table 2). Sediments from RO, SD1, and SG1 had similar profile of alkyl-PAH components (Fig. 1a, 1b, and 1e). For example, dibenzothiophene (DBT; C2 and C3) was most abundant in RO (60.0%), SD1 (66.8%), SG1 (63.3%), and SG3 (47.5%), but fluorene (FLU; C3) was the major component in SD2 and EH1; 50.1 and 83.0%, respectively (Fig. 1c and 1d). Naphthalene (NA) represented <10% among total alkyl−PAHs in sediments of SD1 (4.1%), SD2 (5.2%), SG1 (8.4%), and EH1 (5.3%). SG2 and SG3 also had similar composition of alkyl-PAHs; NA (C3) and DBT (C2 and C3) existed in the largest fraction in the two samples (Fig. 1f and 1g). Overall, RO, SD1, and SG1 had similar PAH composition profile that was distinct from SD and EH1. SG2 and SG3 shared a PAH composition profile that was intermediate of the two groups.

In the present study, the specific components of alkyl−PAHs varied among samples (Table 2), even among the sediments collected from the same site. Natural weathering process can be highly variable, depending on the wave energy level at the shoreline, the extent and characteristics of exposed oil on the shoreline, and the patchy distribution of residual oils in the sediments (Taylor and Reimer, 2008; Hong et al., 2015). In general, however, compared to the sediment extracts sampled previously from the same locations (Yim et al., 2011; Hong et al., 2012; Lee et al., 2013), the samples collected in the present study showed clear indications of weathering, such as decreased fraction of lower molecular weight PAHs. In marine environment, saturates and aromatics of crude oils can be easily biodegraded (Wang et al., 1998; Harayama et al., 1999). For example, PAHs such as NA and phenanthrene (PHE) are readily degraded aerobically, and their fractions are lower in the sediments contaminated with weathered oil. Therefore, the ratios

Table 1
Concentrations and percentages of parent−PAHs and alkyl−PAHs in sediments samples.

Sites	Concentrations of parent-PAHs (ng/g dw)	%	Concentrations of alkyl−PAHs (ng/g dw)	%
RO	4.29	0.45	957.84	99.55
SD1	0.16	1.51	16.08	98.49
SD2	0.10	3.21	1.15	96.79
SG1	2.95	0.96	220.11	99.04
SG2	1.02	8.18	66.60	91.82
SG3	1.33	1.32	40.21	98.68
EH1	0.03	8.18	0.39	91.82

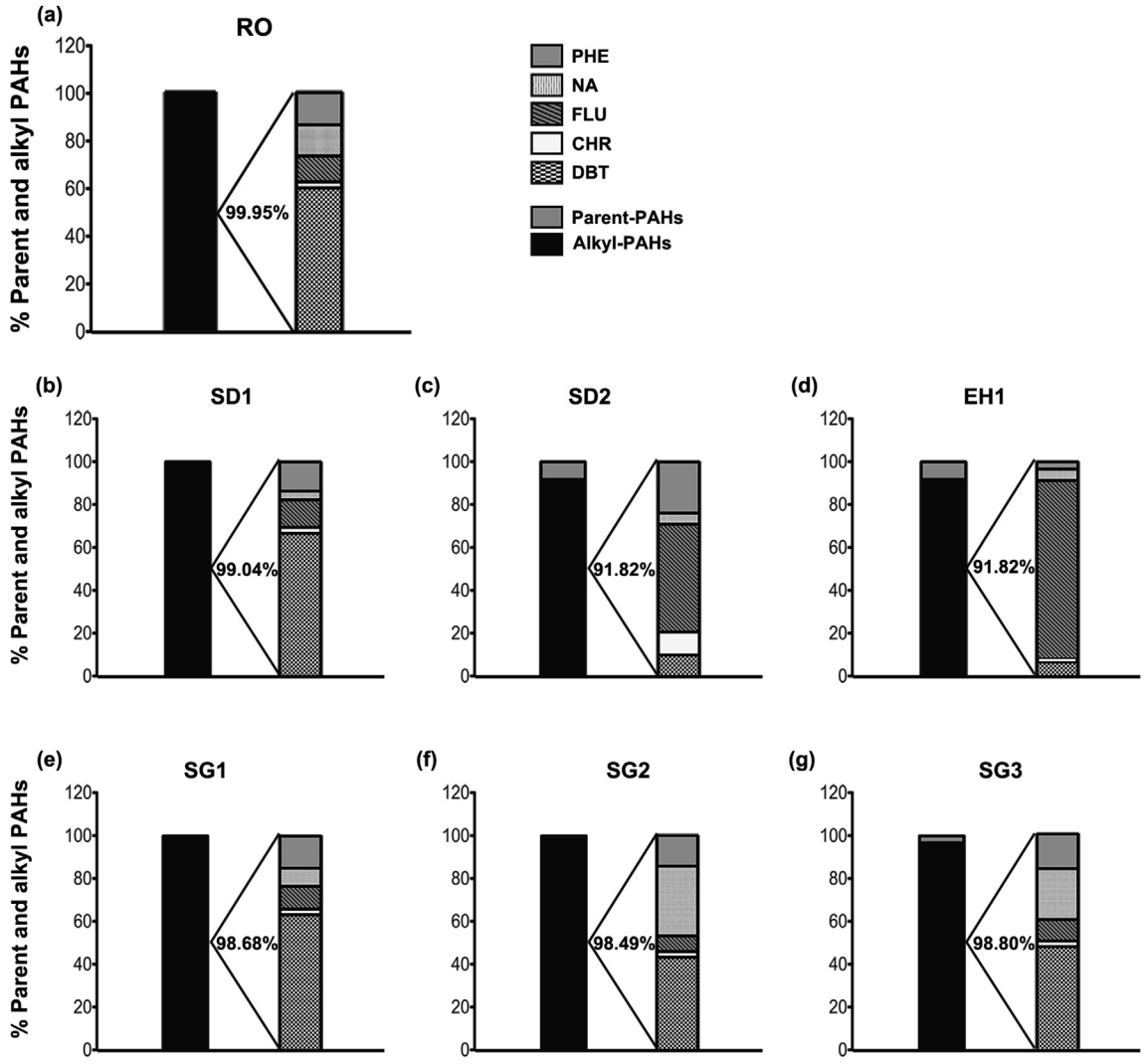

Fig. 1. Profiles of parent–PAHs and alkyl–PAHs of (a) RO (residual oil), (b) SD1, (c) SG1, (d) SG2, (e) SG3, (f) SD2, and (g) EH1. Left bar shows proportion between parent-PAHs and alkyl-PAHs. Rightbar shows proportion of analyzed chemical compositions in alkylalkyl-PAHs. DBT = dibenzothiophene; CHR = chrysene; FLU = fluoranthene; NA = naphthalene; PHE = phenanthrene.

Table 2
Concentrations (ng/g) and percentages (in parentheses) of five major alkyl–PAHs in sediment samples.

Compounds	RO	SD1	SD2	SG1	SG2	SG3	EH1
Naphthalene	124.28 (13.0)	0.65 (4.1)	0.06 (5.2)	18.47 (8.4)	21.60 (32.4)	9.52 (23.7)	0.02 (5.3)
Dibenzothiophene	574.76 (60.0)	10.74 (66.8)	0.12 (10.2)	139.37 (63.3)	28.83 (43.3)	19.12 (47.5)	0.03 (6.9)
Fluorene	104.53 (10.9)	2.06 (12.8)	0.58 (50.1)	23.44 (10.6)	4.85 (7.3)	4.00 (10.0)	0.32 (83.0)
Phenanthrene	129.81 (13.0)	2.18 (13.5)	0.27 (23.8)	33.06 (15.0)	9.53 (14.3)	6.50 (16.2)	0.01 (3.4)
Chrysene	24.46 (2.6)	0.45 (2.8)	0.13 (10.8)	5.78 (2.6)	1.79 (2.7)	1.07 (2.7)	0.01 (1.4)

of differentially alkylated DBT and PHE or chrysene (CHR) (Schmitz et al., 1996) are frequently used to estimate the extent of weathering of sediments (Yim et al., 2011; Hong et al., 2012, 2015).

Chemical analysis revealed that sediment quality in the study area did not fully recover even 7 years after HSOS. In addition, total PAH concentration, as well as the weathering patterns and profiles of alkyl–PAHs were different from sample to sample. Therefore, both the quantity (total PAH concentration) and quality (PAH composition) should be considered when characterizing environmental samples.

3.2. AhR-binding affinity

The % $TCDD_{max}$ values of RE of sediments from the H4IIE–*luc* bioassay are presented in Fig. 2. RE of RO and SD1 elicited 105.9% and 62.9% of the responses, respectively. The % $TCDD_{max}$ was calculated at 24.3% for RE of SD2. SG1 and SG2 induced 95.1% and 97.0% of the $TCDD_{max}$, respectively, but 3.0% and 1.8% $TCDD_{max}$ were measured in SG3 and EH1 sediments respectively. The higher % $TCDD_{max}$ values measured from the extracts of RO, SG1, and SG2 samples are likely due to the greater concentrations of total PAHs. This result is similar to Hong et al. (2012), which reported that

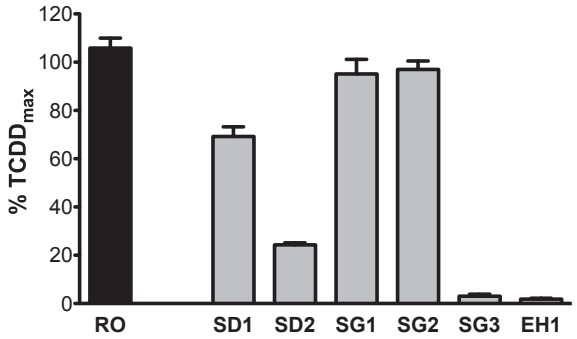

Fig. 2. Response of H4IIE-*luc* bioassay to raw extracts of SD1, SG1, SG2, SG3, SD2, and EH1. Responses are expressed as % TCDD$_{max}$. Data are shown as mean ± standard deviation.

subsurface sediments, which had greater concentrations of oil, displayed greater AhR–mediated potencies. In addition, they found that among sediment samples, weathered fractions that contain greater fractions of alkyl–PAHs induced greater AhR activity.

Nevertheless, total amount of PAHs cannot fully account for the % TCDD$_{max}$ values observed in the present study. For example, SD1 and SD2 samples showed higher % TCDD$_{max}$ values compared to SG3, even though the total amount of both parent and alkylated PAHs of SG3 were higher than SD1 and SD2. Therefore, our results imply that the composition of the chemicals, or their interactions, may also play an important role in inducing AhR-mediated responses.

3.2.1. AhR–mediated potencies of fractionated samples

The F2 fractions generally induced the greatest % TCDD$_{max}$ (0.2–107.3%), followed by F3 fractions (0.4–78.5%) (Fig. 3). However, H4IIE–*luc* responses were calculated at <2.4% in F1 and F4 fractions. These results are consistent with those of previous studies that reported higher binding affinities of aromatic compounds, including parent–PAHs and alkyl–PAHs to AhR (Barron et al., 2004; Larsson et al., 2012; Hong et al., 2016). The results of the present study confirm that the responses of AhR–mediated potencies are mostly caused by the aromatic compounds associated with oil contamination.

Interestingly, the % TCDD$_{max}$ of fractionized samples in SG2 were calculated to be quite low, i.e., 0.82, 5.31, 1.47, and 0.09%, for F1, F2, F3, and F4 respectively, even when the % TCDD$_{max}$ of the RE was 96.99%. AhR–mediated potencies of SD2 showed similar pattern to those of SG2, i.e. AhR binding affinity was dramatically reduced in the fractionated samples, when compared to the RE (Figs. 2 and 3). These observations might be due to interactions among the components. Crude oil consists of diverse mixtures of chemicals such as PAHs and alkylated PAHs, heavy metals, and others. These diverse chemicals can interact with one another and often induce synergistic toxicological effects. Wassenberg and Di Giulio (2004) reported that PAHs such as α-naphthoflavone (ANF) and β-naphthoflavone (BNF), synergistically enhanced the deformity of killifish embryos induced by benzo(*a*)pyrene (BaP). On the other hand, chemical interaction may result in antagonistic responses. For example, Kim et al. (2014) reported that fractionation of sediment samples with high estrogenicity resulted in loss of estrogenicity, as measured by the H295R cell line assay. Koh et al. (2002) also reported that fractionized extract yielded greater responses than the corresponding raw extract of sediment sample. These results suggest that effect directed analysis (EDA) using fractionated samples often cannot reflect true picture of toxicological profile of the sample, and that mixture effects have to be considered when assessing the toxic potencies of environmental samples (Hong et al., 2016).

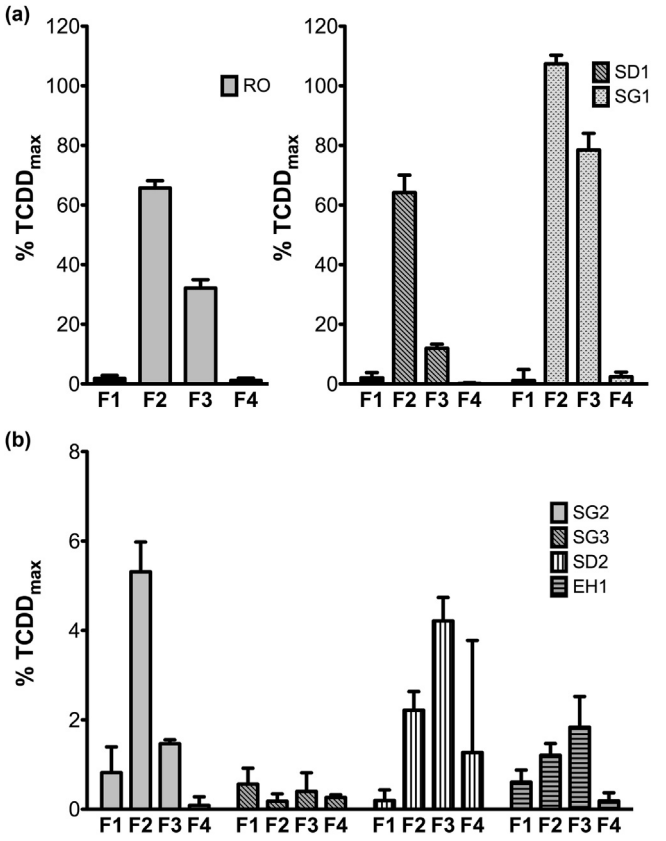

Fig. 3. Response of H4IIE-*luc* bioassay to silicagel fractions of extracts from (a) RO, SD1, SG1, and (b) SG2, SG3, SD2, and EH1. Responses are expressed as % TCDD$_{max}$. Data are shown as mean ± standard deviation.

3.3. ER-binding affinity

While the % E2$_{max}$ results varies among different sampling sites, there was no noticeable induction of ER–dependent responses (Fig. 4). In the present study, all samples induced less than 50% E2$_{max}$; the greatest % E2$_{max}$ of RE was observed in RO (41.3%), followed by SG3 (25.8%), and SG1 (13%). Previous studies revealed that PAHs can disrupt sex hormone homeostasis (Vrabie et al., 2010; Xia et al., 2011). Vrabie et al. (2010) showed that oils were able to induce estrogenic responses in estrogen receptor β (ERβ) in yeast estrogen screening assay (Vrabie et al., 2010). Similarly, Ji et al. (2011) found that the sediment extracts from HSOS induced significant increase of E2 and decrease of testosterone concentrations, as well as changes in the expression of major steroidogenic mRNAs such as *cyp19* and *cyp11b2*, in human adrenal (H295R) cells.

In the fractionized sediment samples, however, MVLN–*luc* responses showed inconsistent patterns. For example, the greatest induction was observed in the F2 fraction rather than RE, in samples like SD1, SD2, SG1, and EH1 (Fig. 4a). However, RE induced higher responses than the fractions, in RO, SG2, and SG3 samples (Fig. 4b). Similar results were also observed in previous studies (Koh et al., 2002; Vrabie et al., 2012; Kim et al., 2014). For example, Kim et al. (2014) reported no significant responses from the RE, while its fractionized extracts exhibited strong estrogenicity (Kim et al., 2014). In another study, significant response in ER–mediated potency was observed in only 1 out of 22 RE samples, while 12 out

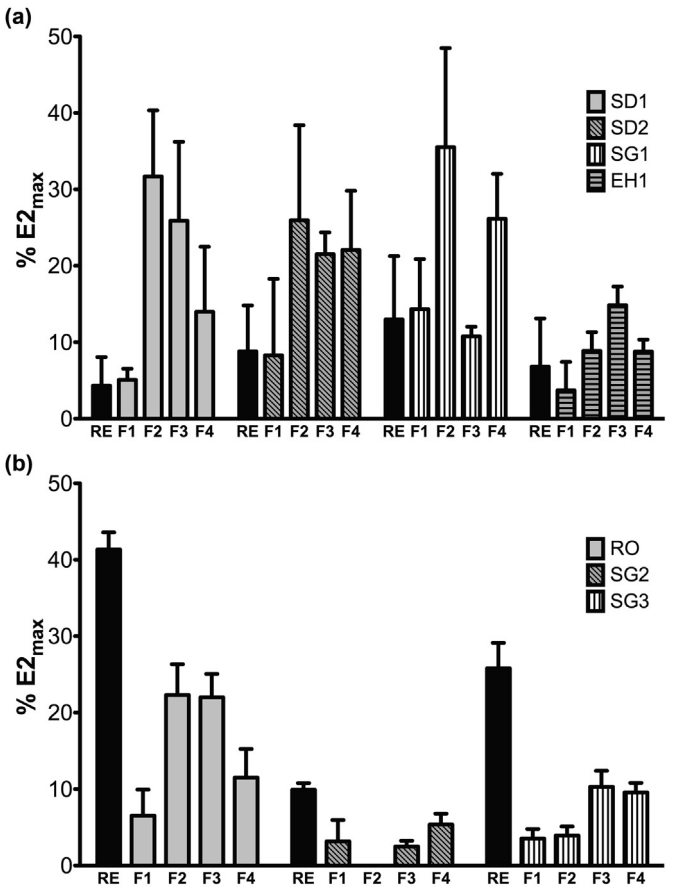

Fig. 4. % $E2_{max}$ of raw extracts and silicagel fractions f (a) SD1, SD2, SG1, and EH1, and (b) RO, SG2, and SG3 samples. Black bar shows % $E2_{max}$ of raw extracts of sediments, and gray bar shows % $E2_{max}$ of silica gel fractions. Data are shown as mean ± standard deviation.

of 15 F1 fractions and 13 out of 15 F2 fractions showed significant estrogenic responses (Koh et al., 2002). Although the reason for this inconsistency in ER–mediated potencies in each fractionized samples is not clear, these results clearly indicate interactions among components of oil remnants that induce antagonistic or agonistic effects. Components of F3 (polar and resins) can induce estrogenicity because compounds in F3 are linked to known phenolic chemicals such as nonylphenols (NPs) and BPA (Kim et al., 2014), but in the present study, F3 fractions showed little effects on ER–mediated potency (25.9% or less). In contrast, F2 fractions containing aromatic compounds elicited higher responses in some samples (Fig. 4). Therefore, in our study which lacks analytical data for chemicals except PAHs, it is difficult to support that estrogen receptor antagonists caused the ER-mediated potency of the sediment extracts. Considering the results of previous results, it may be possible that weathered oil components include chemicals that induce steroidogenic effects that act independently from ER.

The results of the present study show that AhR-binding and ER-binding potencies measured in the sediment samples differed. Our results showed that the sediment samples exhibited relatively high AhR binding affinity, but relatively low ER binding affinity. It is known that inhibitory crosstalk exists between AhR and ER. (Safe and Wormke, 2003; Matthews and Gustafsson, 2006). However, our observations cannot be solely explained by this inhibitory crosstalk. This is because we have used cell-based assays that may preclude the influence of other receptor-mediated activities. Therefore, it would be prudent to conclude that the AhR binding potencies and ER binding potencies measured from the sediment extracts in the present study are independent. Our observation may simply reflect the toxicological characteristics of the composition of contaminants that are present in the sediment samples.

3.4. Multivariate analysis (principal component analysis)

The H4IIE–*luc* bioassay results revealed that AhR binding affinity varied greatly among different sediment samples (Figs. 2 and 3). Therefore, PCA was performed to identify the major PAH components that may characterize the composition of sediment samples (Fig. 5). Of the five most abundant alkyl-PAHs (DBT, PHE, NA, FLU, and CHR), DBT was the main factor that clustered RO, SD1, and SG (SG1, SG2, and SG3) samples, but FLU was the main factor that associated with SD2 and EH1. This result was somewhat predictable from the relative concentrations of the different chemical components (Table 2, Fig. 1). However, when we overlayed the H4IIE-*luc* assay results, we observed that this clustering result did not correlate well (color-coded). Similar inconsistency was observed MVLN assay as well (data not shown).

Our observation suggests that both the total amount of alkyl–PAHs and their compositions are more important factors in inducing AhR–mediated and ER-mediated potencies in oil contaminated sediments. However, neither the total PAH content nor the concentration of major components could independently explain AhR-mediated potency and ER-mediated potency. It is extremely challenging to identify specific chemicals that act as AhR or ER agonists from the oil contaminated sediments with complex chemical profiles. Compositional changes of PAHs that occur during weathering processes makes it even more difficult, as products of the weathering process will have different modes of actions.

One caveat in this study is that only the PAH content was measured from the oil contaminated sediments. Therefore, the influences of other oil components, such as nitrogenous compounds, other hydrocarbons including alkanes and asphaltics, or metals (Yim et al., 2011), were not considered, although such components may not represent major parts of the oil residuals that present in the sediment samples of the present study. In addition, with bioassays that have been utilized to capture PAHs related toxicity of the oil, we found that EDA with fractionized environmental samples may not properly elucidate the toxicological effects of the oil contamination, possibly because of interactions of complex chemicals in the sample.

4. Conclusions

In the present study, AhR–mediated potencies were still detected in the coastal sediment samples collected seven years after HSOS. We have confirmed that total PAHs decreased over time in certain areas. In addition, compared with previous reports from the same sites, both AhR–mediated and ER–mediated potencies have decreased. EDA results suggest that aromatics, resins and polar compounds of oil contaminated sediments can induce huge AhR–mediated responses, but limited ER–mediated responses. Bioassay results from the fractionated samples did not match those observed from RE, implying that interactions among the oil components exist. Such mixture interactions should be considered when conducting EDA of petroleum-derived environmental samples, as too much fractionation may result in wrong representation of the sediment toxicity. More detailed studies that combine mechanism-based bioassay tools and chemical analysis on fractionated samples are necessary to fully understand the biological effects induced by oil residues from oil spills. Such understanding will provide basis for selection of appropriate risk assessment

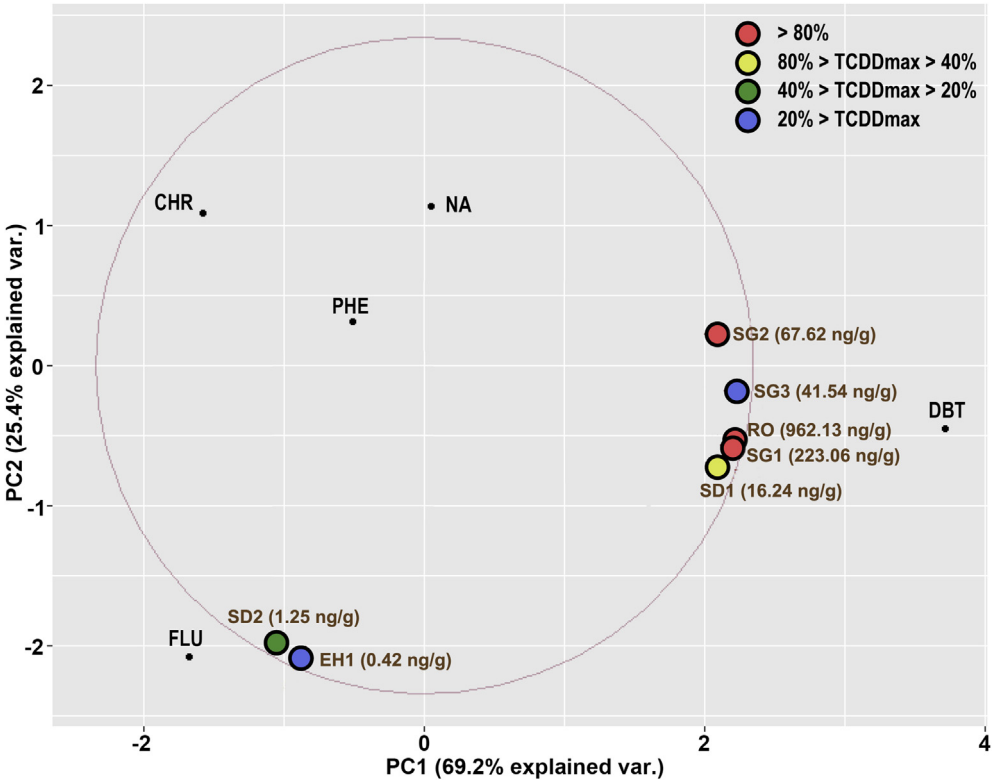

Fig. 5. Result of principal component analysis (PCA) of sampling sites. The sites were grouped into two major clusters according to their chemical components, alkylated PAHs. DBT (dibenzothiophene) was a main factor that clustered RO (Residual oil), SD1, SG1, SG2, and SG3, and FLU was the other main factor that clustered SD2 and EH1. % $TCDD_{max}$ of each sample is represented by different colors, and did not correlate with clustering result. DBT = dibenzothiophene; CHR = chrysene; FLU = fluoranthene; NA = naphthalene; PHE = phenanthrene.

measures, and for prioritizing options in the risk mitigation and remediation processes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.10.078.

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Reconnaissance of dioxin-like toxicities in sediments of Taean, Korea-Seven years after the *Hebei Spirit* Oil Spill

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Contents of Supporting Information

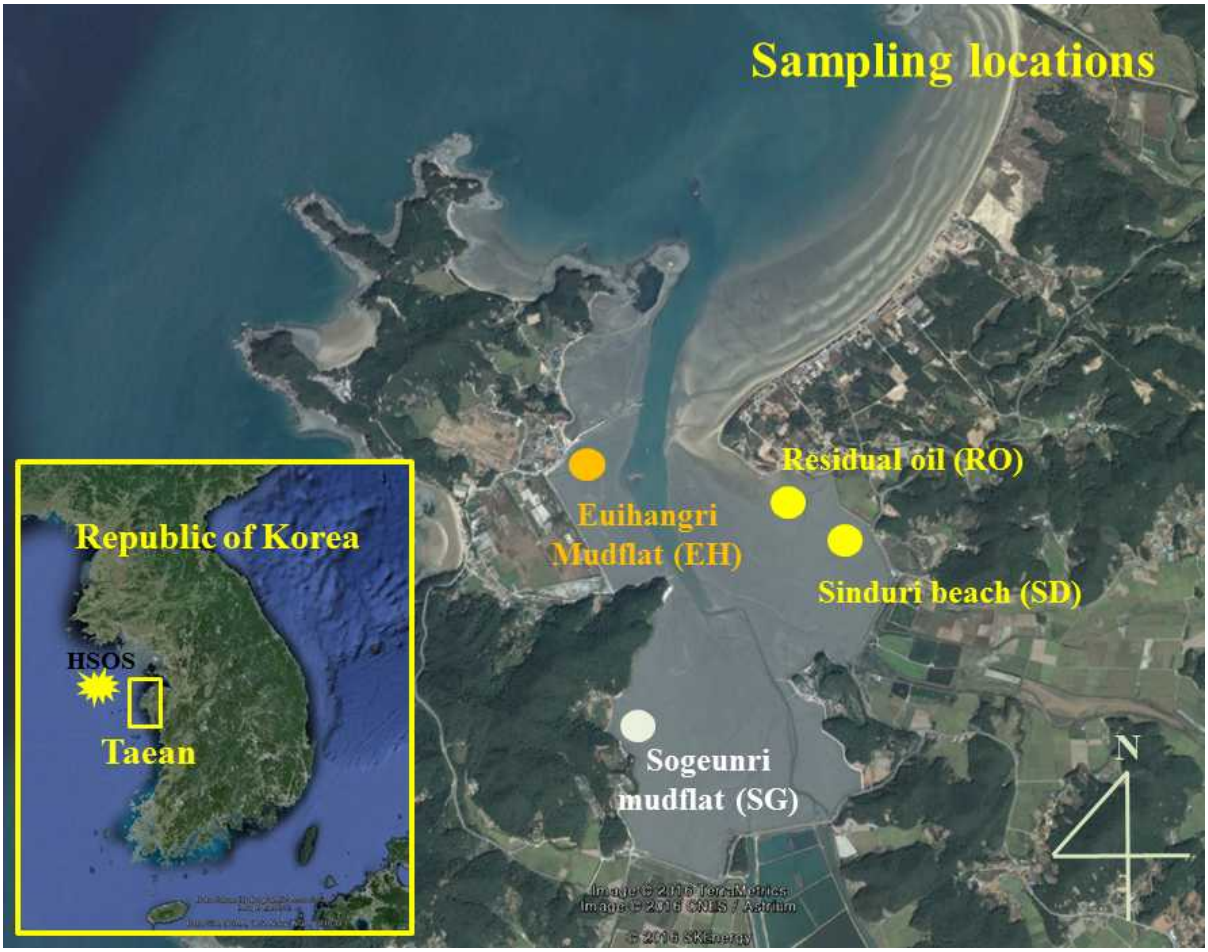

Figure S1. Location of sampling sites, RO (Residual oil), SD (Sinduri beach), SG (Sogeunri mudflat), and EH (Euihangri Mudflat). (Satellite photo provided by Google Earth version 7.1.5. 2015. Image providers are represented in the bottom of each map: Big map was provided by Image © 2016 TerranMetrics; Image © 2016 CNES / Astrium. Inset map was provided by Data Japan Hydrographic Association; Image Landsat; Data SIO, NOAA, U.S. Navy, NGA, GEBCO).

Table S1. Sampling location, sampling date, and sediment type.

Location	Sample ID	Sampling date	Sediment type
Sinduri	RO	Jun. 2014	Sand, Residual oil detected
	SD1	Oct. 2014	Sand, residual oil (RO) detected previously
	SD2	Oct. 2014	Sand, backshore zone of SD1
Sogeunri	SG1	Oct. 2014	Mudflat
	SG2	Oct. 2014	Mudflat
	SG3	Oct. 2014	Mudflat
Euihangri	EH1	Oct. 2014	Mudflat